APPENDIX A

RECORD AND DISCLOSURE OF INVENTION

NC 82197 NMR 876

FOR USE BY NAVY INTELLECTUAL PROPERTY OFFICE

DATE DISCLOSURE RECEIVED	NAVY CASE NO.

INSTRUCTIONS: A Navy employee should use this form when submitting an invention disclosure to the Department of the Navy. Fill in each blank with the requested information or enter "NONE" as appropriate. Original and two copies should be printed or typed and forwarded to the intellectual property office responsible for providing services to your activity. Where space on this form is inadequate, enter "see attached page," use plain pages as needed, and identify item by number. When completely executed, this form becomes an important legal document useful in proving priority of invention. This form may also be used by a contractor or grantee for disclosing an invention to the Navy.

PART 1. RECORD OF INVENTION

INVENTOR(S)	ADDRESS	POSITION TITLE	EMPLOYER (activity & Code No., or Company address)
Johnny Dale Callahan	1815 Graybird Ct. Severn, MD 21144	Contractor-VRDD (50%) Contractor BDRD (50%)	Viral and Rickettsial Disease Program Biological Defense Research Program NMRC
Joseph John Temenak	718 Fenton St. Takoma Park, MD 20912	Chief Microbiologist, NMRC Rickettsia Diseases Program	US Army, Active Duty

DESCRIPTIVE TITLE OF INVENTION

Serotype Specific Fluorogenic Probe based PCR (TaqMan) Assays Against the C and NS5 Genomic Regions of Dengue Virus.

3. CONCEPTION, INITIAL RECORDS AND RESULTS OF FIRST MODEL

a. EARLIEST DATE AND PLACE INVENTION WAS CONCEIVED (Identify persons and records to support date and place)

Inventors met at the Naval Medical Research Center, Bethesda, MD. The concept was discussed in March 99, supplies were ordered and the first experiment took place on 7 April 1999.

b. DATE AND PRESENT LOCATION OF FIRST SKETCH, DRAWING OR PHOTO AND FIRST WRITTEN DESCRIPTION (Such as notebook entries etc.)

First notebook entry describing the concept was on 29 March 1999 at the Naval Medical Research Center.

c. DATE AND PLACE OF COMPLETION OF FIRST MODEL, PROTOTYPE, PRELIMINARY SYNTHESIS, FORMULATION, ETC., AND ITS PRESENT LOCATION

First model completed and tested on 2 Aug 1999 at the Biological Defense Research Program, Bldg17 laboratory NMRC, using the TaqMan Instrument. Formulation found in TaqMan PCR notebook #1, page 38, Located at the Viral and Rickettsial Disease Divisin

d. DATE AND PLACE OF FIRST TEST OR OPERATION AND THE RESULTS (Give name and address of witnesses, and present location of records) The date and place of first test was the same as in "part c." above. The results showed that the test was specific for the target and did not cross-react with related viruses. Witness was Capt. Joseph Temenak., location of records is the same as in "part c." above.

4. OTHER RECORDS (Notebook entries, descriptions, reports, drawings, etc.)

IDENTIFICATION	DATE OF DOCUMENT	PRESENT LOCATION
Notebook titled: Taqman Assay Development #2	3/1/99	Rm 3N6D, WRAIR/NMRC

5. OTHER INDIVIDUALS TO WHOM INVENTION WAS DISCLOSED

NAME	ACTIVITY OR COMPANY INDIVIDUAL REPRESENTS	DATE DISCLOSED	TYPE (oral or written disclosure)
Cutis B. Hayes	Viral and Rickettsial Disease Program, NMRC	2 Aug 1999	Oral
Capt Al Matoczun	Biological Defense Research Program, NMRC	2 Sept 1999	Oral

Name of Invention: Serotype Specific Dengue Virus Fluorogenic Probe based PCR (TaqMan) Assays Against the C and NS5 Genomic Regions.

Inventors: Callahan, Johnny Dale
Temenak, Joseph John

Purpose.
The purpose of the assay is to provide real time quantitative measurements of dengue virus in research samples.

Background
The dengue viruses are a major public health concern with serious medical and economic consequences and are currently considered the most important arthropod disease affecting humans in terms of morbidity and mortality.[1,2]

Previous methods of quantitating dengue viremia involved the isolation of virus from samples using tissue culture, IFA, and plaque titer methods. These classical methods are considered the gold standard, however, these methods are tedious, slow, and often difficult to standardize, and require specialized expertise. The total turn-around time is often two to three weeks and the isolation rates and sensitivity are low.

Description of Operation
Recent advances in the molecular biology and especially nucleotide sequencing of arthropod borne viruses have enabled comparisons to be made of sequences representing numerous flaviviruses.[3] Sequence alignments are a powerful tool enabling the design of very specific and sensitive assays for the detection of dengue viral RNA. By aligning multiple sequences representing not only serotypes of dengue but also including topotypes or genotypes within serotypes, very specific assays can be designed that can detect a given serotype across multiple geographic regions.

The assays described here are a group of four fluorogenic probe-based PCR (TaqMan) assays for the detection of Dengue virus types 1,2,3 and 4. The assays specifically targets the NS5 genomic region of dengue virus type 1, and C genomic regions of dengue virus types 2,3 and 4 (Table 1).

Type specific oligonucleotide primers and fluorogenic probes were designed against the NS5 and C regions of the dengue genome. RNA was extracted from test samples using the Qiagen QIAamp viral RNA kit. The PCR assay consisted of a 30 minute RT step (60°C), linked to a 15 second melting step (95°C), and 45 cycles of PCR at temperatures based on the calculated Tm of the primers used (95°C and 60°C). Primers and probe sets were used to test panels containing extracted viral RNA from several strains each of dengue 1, 2, 3, and 4, and other flaviviruses including Japanese encephalitis, and yellow fever.

Using the Perkin Elmer 7700 instrument, assay specificity was evaluated by testing serotype specific probe and primer sets against specificity panels that included dengue-1,

detection of specific PCR products was determined by monitoring the increase in fluorescence of a dye-labeled oligonucleotide probe.

Advantages and New Features

1. The assay is much more sensitive than the gold standard.
2. The turn around time for a result is measured in hours rather than weeks.
3. Many samples can be run simultaneously and analyzed in seconds.
4. The precision, accuracy, and reproduciblity of measurements is unmatched by traditional methods.

Alternatives

Alternative uses of this assay include re-optimization of the concentration of reagents, but not the design of the assay for use on the light cycler instrument. This would provide real time qualitative determinations for the presence of dengue virus in remote field conditions.

Contributions by the Inventors

Johnny Callahan - 60%
Joseph Temenak - 40%

References:

1. Trent DW; Manske CL; Fox GE; Chu MC; Kliks SC; Monath TP: The Molecular Epidemiology of Dengue Viruses, Genetic Variation and Microevolution. In: (1989) Applied Virology Research, Vol 2, Virus Variation and Epidemiology (E. Kurstk, Eds). Plenum, NY.
2. Halstead SB: (1988) Science 239, 476-481.
3. Duebel V: The Contributions of Molecular Techniques to the diagnosis of Dengue Infection. In: (1997) Dengue and Dengue Hemorrhagic Fever (eds Gubler DJ, and Kuno G.)

Table 1. Probe and Primer designs. There are two designs.

Direction	mer	Sequence	Name	Target
Design	#1			
Forward	25	aag gac tag agg tta kag gag acc c	DEN-GR-10616F	3' non coding region
Reverse	23	ggc gyt ctg tgc ctg gaw tga tg	DEN-GR-10726R	3' non coding region
Probe 1	27	aac agc ata ttg acg ctg gga gag acc	DEN-1-3-10655-T	3' non coding region
Probe 2	27	aac agc ata ttg acg ctg gga aag acc	DEN-2-4-10655-T	3' non coding region
Design	#2			
Forward	25	agg act aga ggt tak agg aga ccc c	DEN-GR-10617F	3' non coding region
Reverse	21	cgy tct gtg cct gga wtg atg	DEN-GR-10726R	3' non coding region
Probe 3	30	aaa cag cat att gac gct ggg aga gac ca	DEN-1-3-106545-T	3' non coding region
Probe 4	30	aaa cag cat att gac gct ggg aaa gac cag	DEN-2-4-106545-T	3' non coding region

All experiments are documented in notebook #1 and represents work in progress from [illegible] 1999 to the present.

6. IDENTIFY ANY PAST, PRESENT OR CONTEMPLATED USE, SALE, OR PUBLICATION OF THE INVENTION
The invention will be used as a qualitative screening test for the presence of any dengue virus in research samples and/or vaccine challenge studies. The work will be published in abstract form in the upcoming annual meeting of the American Society of Tropical Medicine and Hygiene, 27 Nov 1999. A full manuscript will be prepared and submitted for publication soon thereafter.

7. LIST ANY CLOSELY RELATED PATENTS, PATENT APPLICATIONS AND PUBLICATIONS OF YOUR OR OTHER PERSONS

Our group will also be submitting a separate patent application for a diagnostic assay entitled Serotype Specific Fluorogenic Probe based PCR (TaqMan) Assays Against the C and NS5 Genomic Regions of Dengue Virus.

PART II. DISCLOSURE OF INVENTION

Attach on separate sheets of paper a full and complete description of the invention, using the outline given below.

a. PURPOSE. State the purpose of the invention.

b. BACKGROUND. Describe the old methods, materials or apparatus used to perform the purpose of the invention and give their limitations and disadvantages.

c. DESCRIPTION AND OPERATION. Describe clearly and completely the best mode of the invention and give a detailed description of its operation and use. Sketches, prints, photos, or other illustrations should be attached. In the description, use reference characters to refer to components in attached illustrations.

d. ADVANTAGES AND NEW FEATURES. State the advantages of the invention over the old methods, materials or apparatus described in paragraph b above, and the features believed to be new.

e. ALTERNATIVES. Indicate any alternative methods, materials, or apparatus of the invention.

f. CONTRIBUTIONS BY INVENTORS. If this is a joint invention, indicate what contribution was made by each inventor.

PART III. CERTIFICATION OF INVENTORS

I certify that the invention disclosed herein and in the attached documents is the ☐ *sole* ☑ *joint invention of the undersigned and that statements and answers are true to my best knowledge and belief.*

Date	Signature
10/18/99	John D. Callahan
10/18/99	Joseph J. Tennenak
Date	Signature
Date	Signature

PART IV. CERTIFICATION OF WITNESSES

I certify that the invention described herein and in the attached documents has been disclosed to and understood by me.

Date	Signature	Business Address
18 OCT '99	[signature]	Naval Medical Res. Ctr. Forest Glen, MD
Date	Signature	Business Address
20 OCT 99	A.J. Matezyn	NAVAL MEDICAL RESEARCH CENTER, FOREST GLEN, MD.

PAGE 8/12 * RCVD AT 8/3/2004 9:38:12 AM [Eastern Daylight Time] * SVR:USPTO-EFXRF-1/2 * DNIS:8729306 * CSID:301 295 6480 * DURATION (mm-ss):03-42

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Callahan et al. Examiner: T. Garvey
Serial No. 09/726,345 Group Art Unit: 1636
Filed: December 1, 2000
For: Serotype Specific Fluorogenic Probe-Based PCR (TaqMan) Assays Against the Respective C and NS5 Genomic and 3' Non-Coding Regions of the Dengue Virus

AFFADAVIT UNDER 37 CFR 1.131

Honorable Commissioner of Patents & Trademarks
Washington, D.C. 20231

Sir:

Having been advised of the penalty for false statements under 18 U.S.C. § 1001, We, Johnny Dale Callahan and Joseph John Temenak, being duly sworn, state the following:

1. We are the co-inventors of the patent application identified above and inventor of the subject matter described and claimed therein.
2. Prior to August 1999, the pubblication date of Laue, et al. ('589) used in the 35 USC §102(a) rejection of Claim 18 of the instant application, we conceived the idea of extracting RNA from samples containing Dengue-1,2,3, & 4 virus, then converting this RNA to cDNA by reverse transcription, and then performing RT-PCR with serotype-specific primers and probes for Dengue-1,2,3, & 4 virus in order to detect the presence of the amplification product. The procedure known as TaqMan amplification, which uses specific primers in combination with an internal probe that hybridizes to the region to be amplified, was used to help accomplished this task. See Specification at p.5, line 7 through p.6, line 19.
3. An invention disclosure was filed in due course with the Office of Counsel, Naval Medical Research Center and is attached as Exhibit A, which includes a summary of activities relating to conception and reduction to practice of the instant invention. As outline in this

disclosure, the following activities are delineated: the concept was discussed by us and noted in a laboratory notebook on March 3, 1999; the concept was first described in detail in the laboratory notebook on March 29, 1999; the first experiment took place on April 7, 1999; and the first model was completed and tested on August 2, 1999 and recorded in a laboratory notebook. The conception of the instant invention clearly predates the effective date of the reference used in the 35 USC §102(a) rejection of Claim 18.

4. After receiving a favorable patentability opinion from the Intellectual Property Counsel, a provisional patent application was filed in turn on December 1, 1999. A non-provisional application was subsequently filed on December 1, 2000.

5. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

John D. Callahan

Johnny Dale Callahan

Joseph John Temenak

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Callahan et al.

Serial No. 09/726,345 Examiner: T. Garvey

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Johnny Dale Callahan

Joseph John Temenak

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